FIG.
BY CLASS SUBCLASS

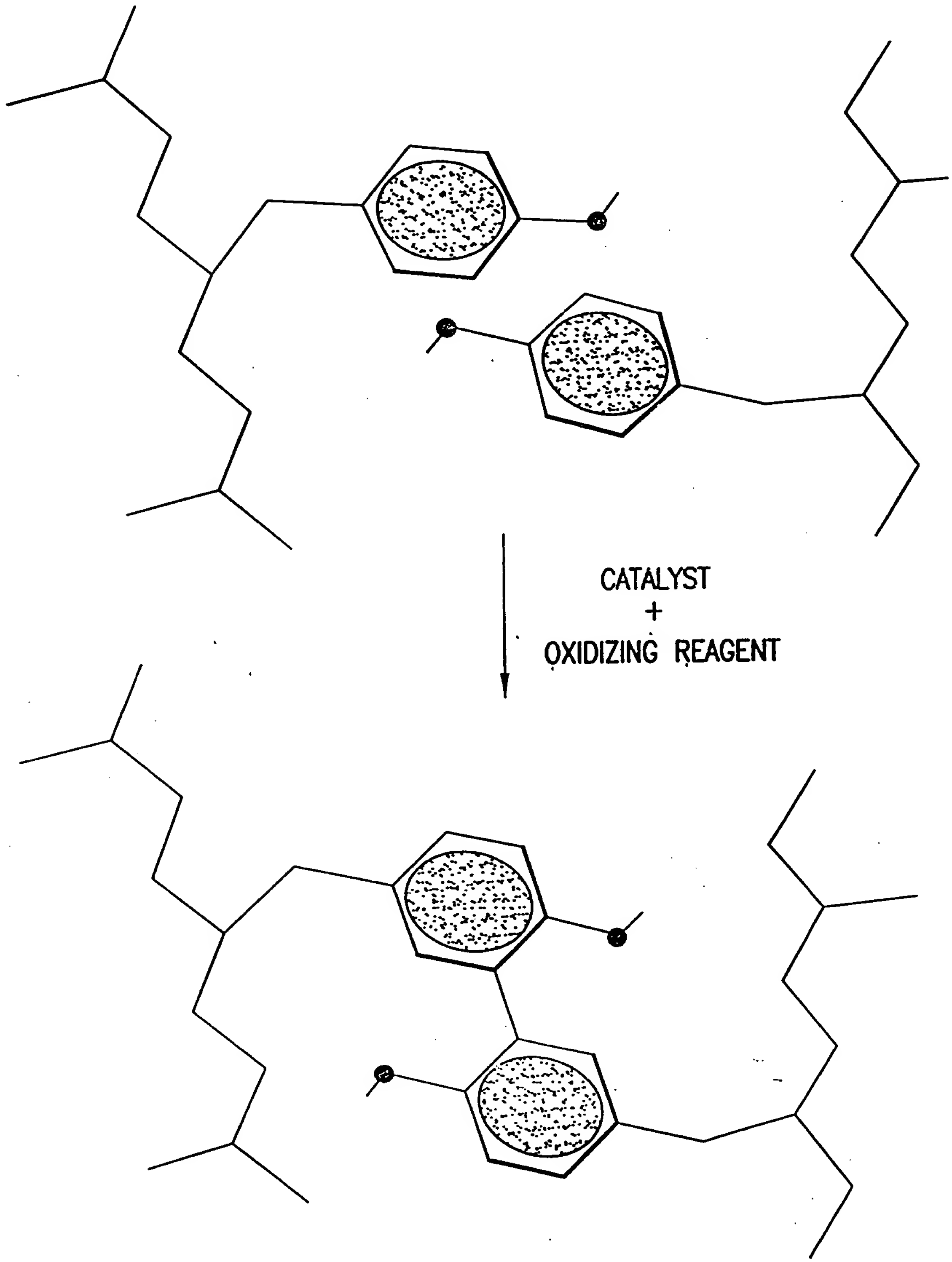

FIG.1A

APPROVED FIG.
BY SUBCLASS
DRAFTSMAN

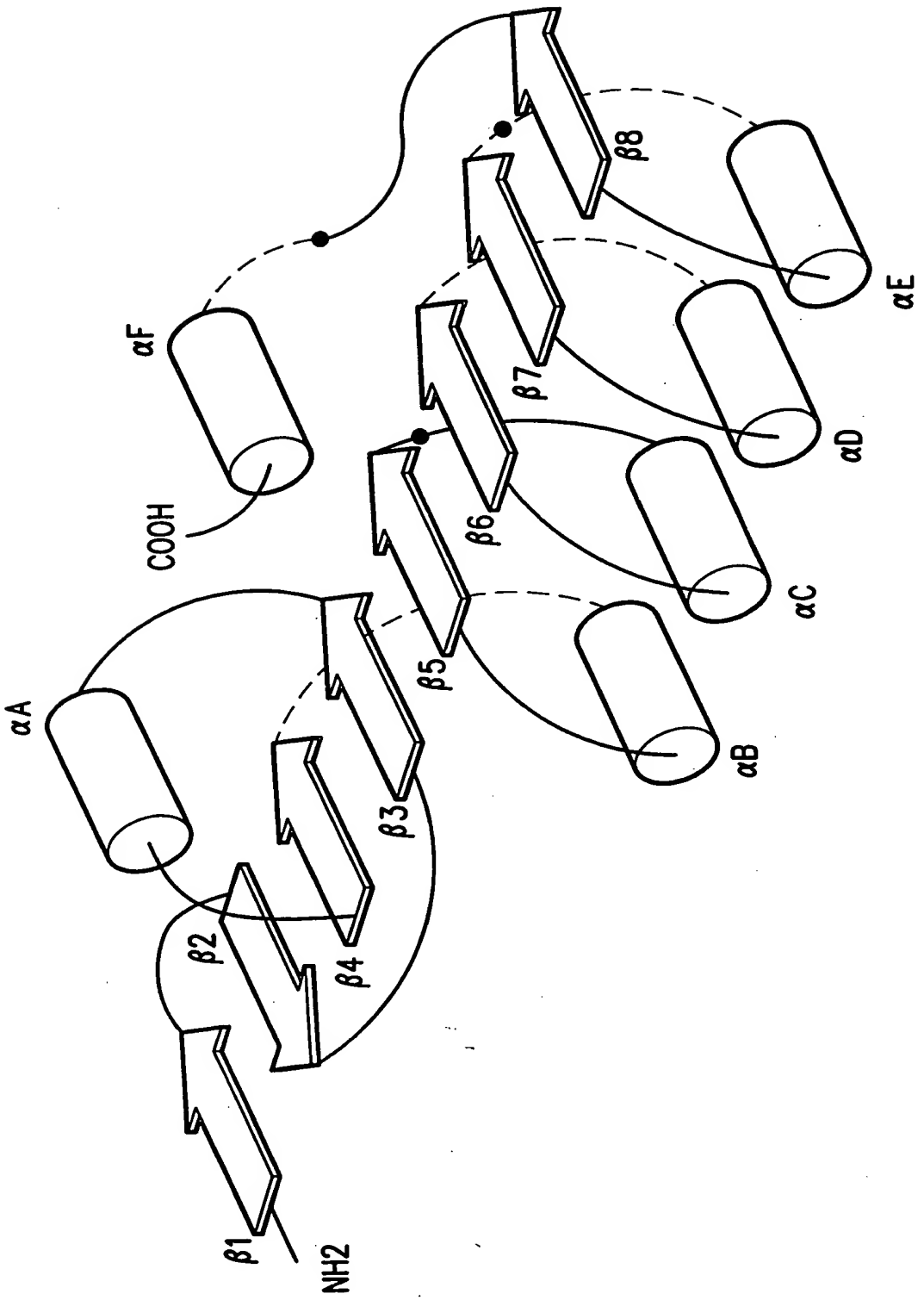

FIG.1B

SUBCLASS

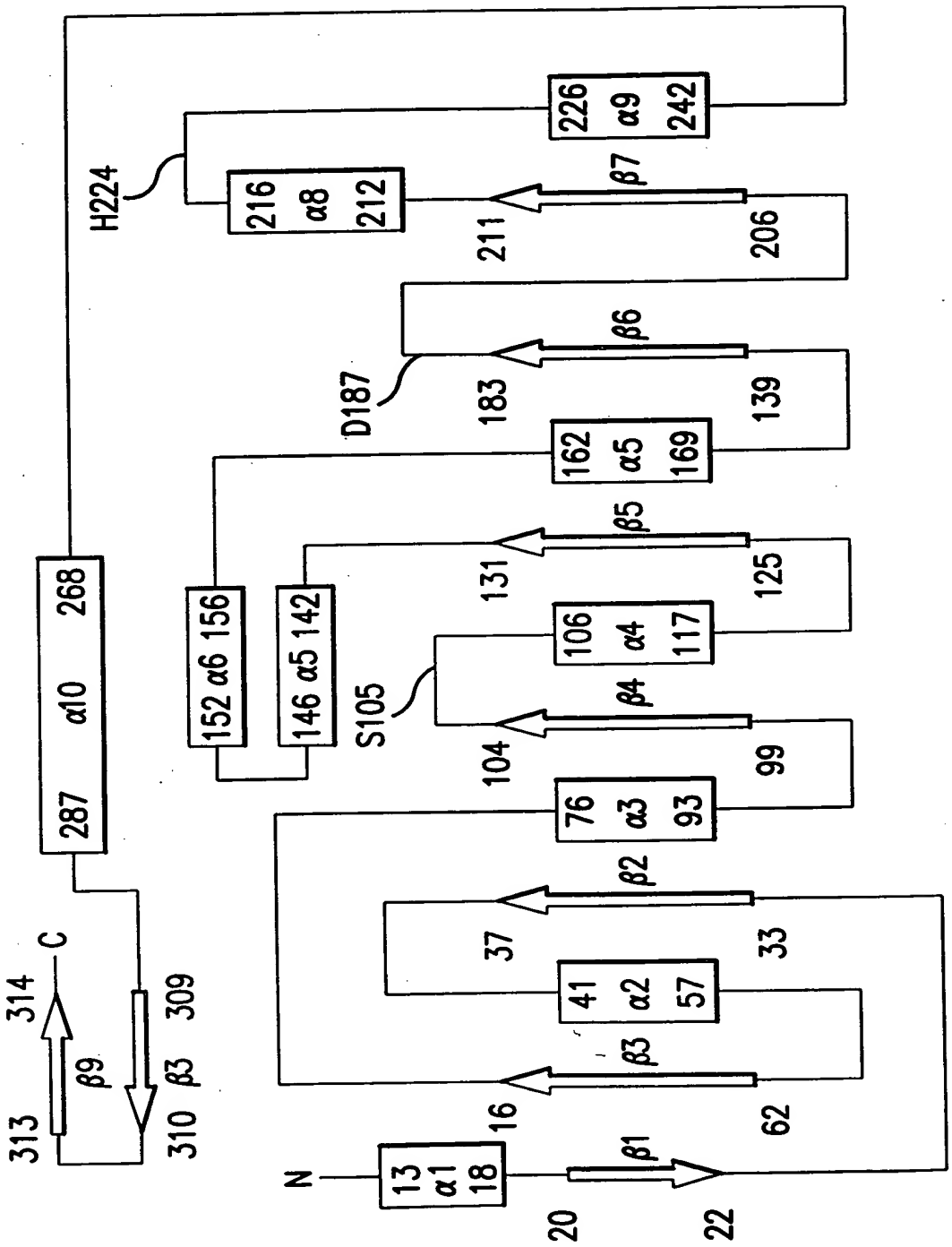

FIG. 1C

APPROVED
BY
DOUGLASS

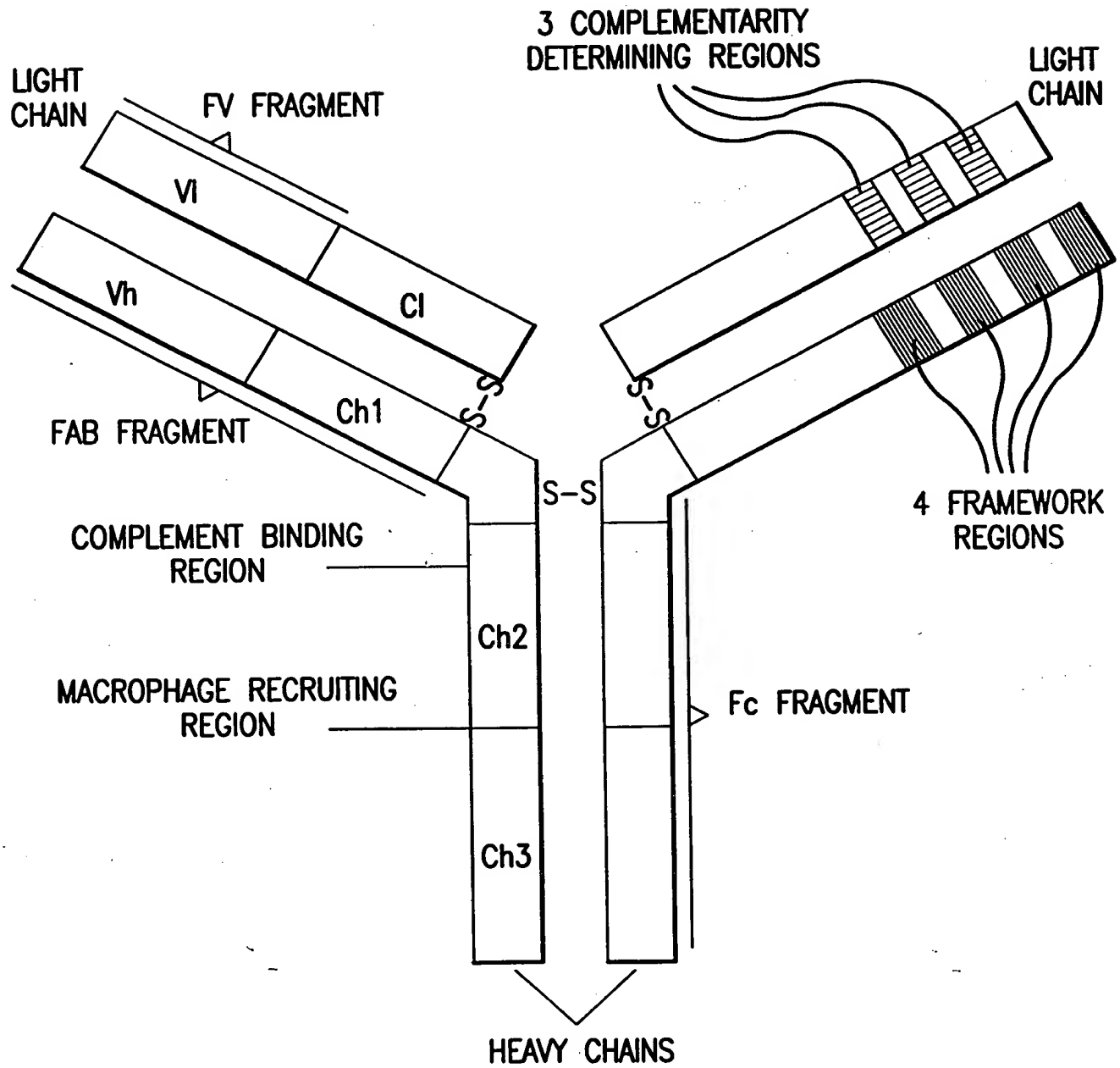

FIG.1D

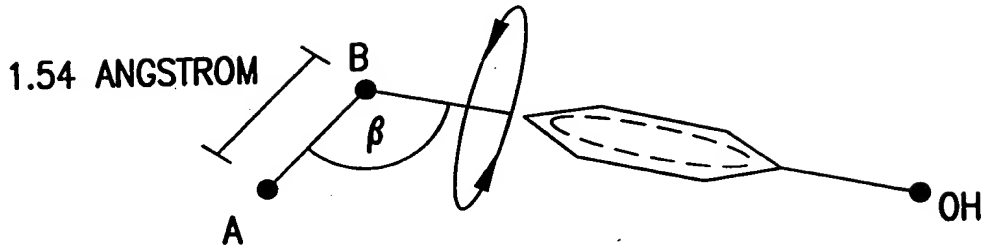

FIG.2A

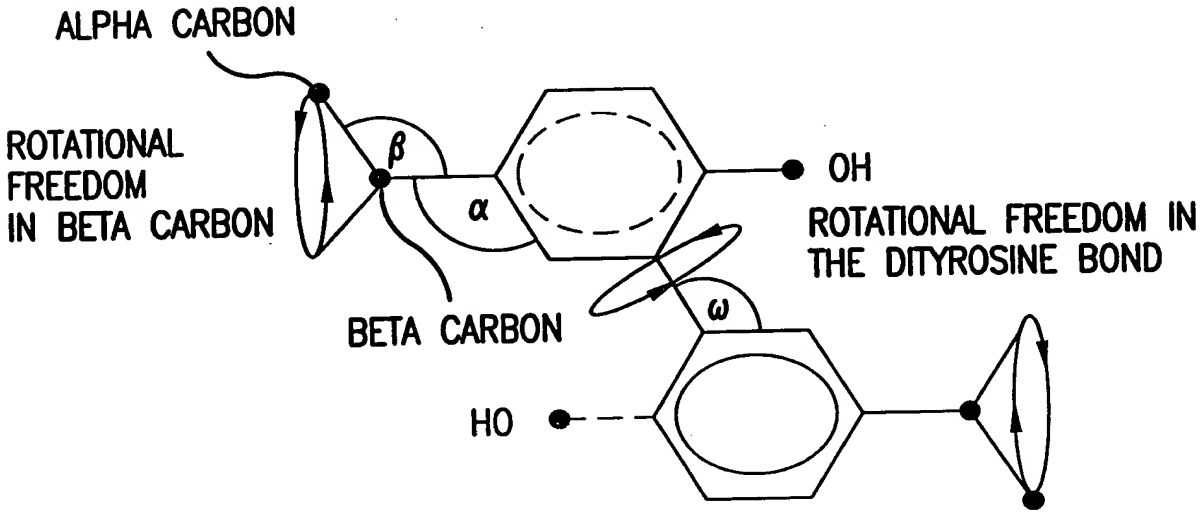

FIG.2B

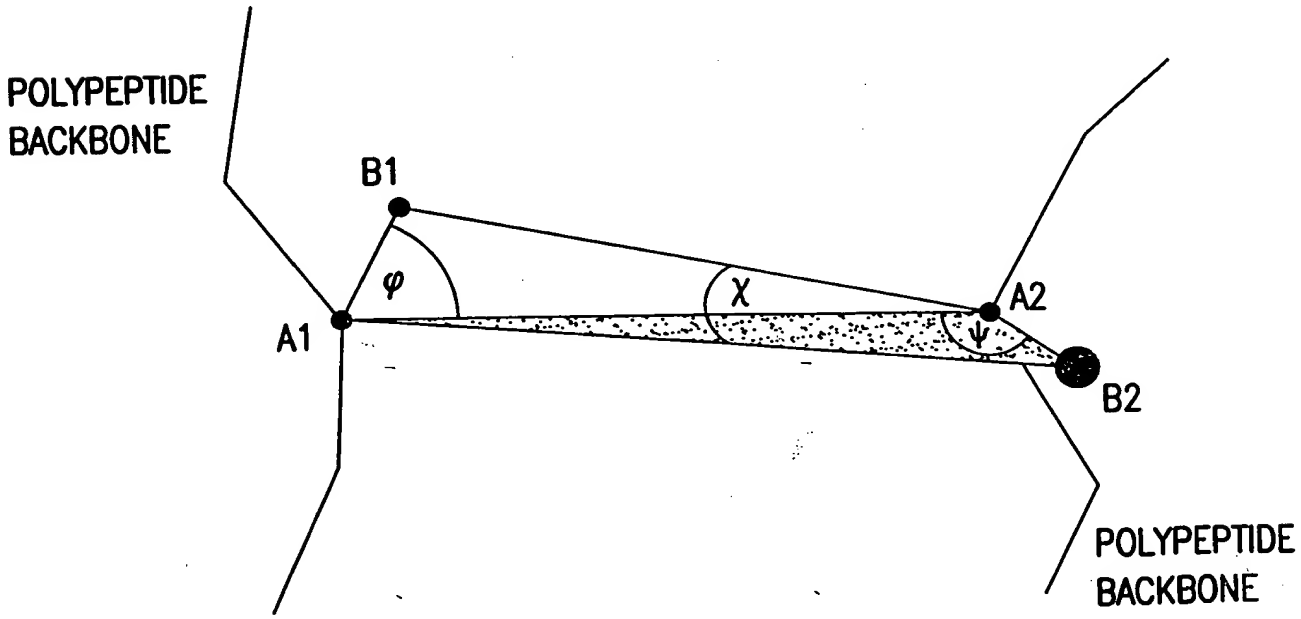

FIG.2C

APPROVED BY DRAFTSMAN
SUBCLASS

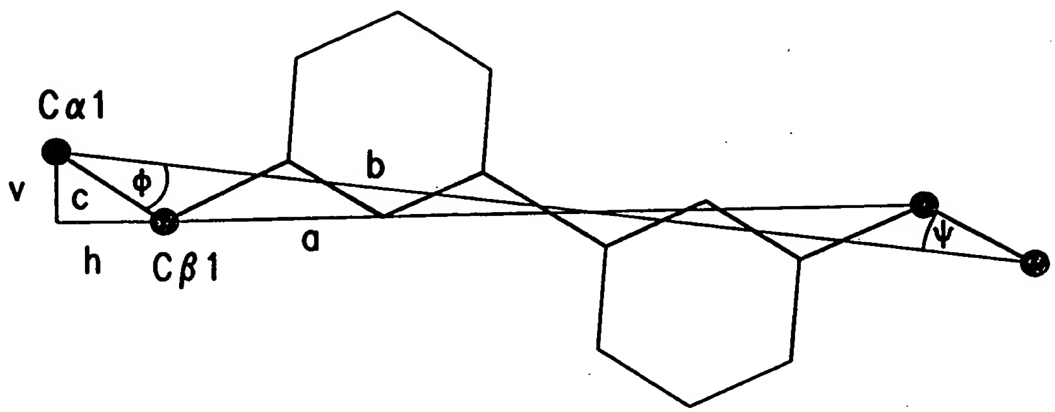

FIG.3A

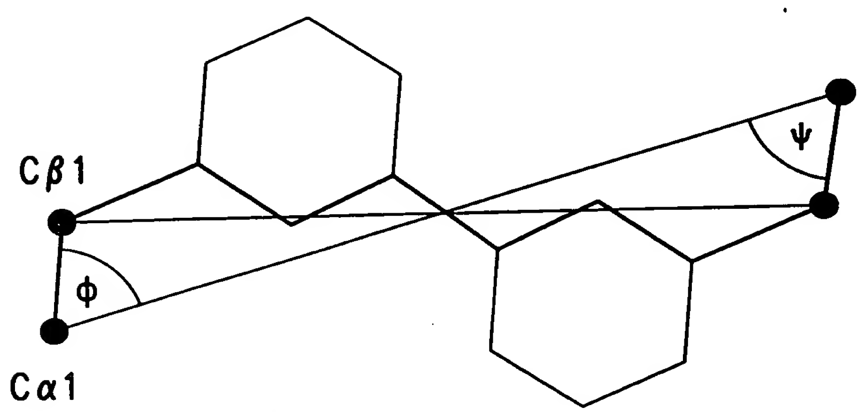

FIG.3B

09837235.041801

AFFIX
BY
DRAFTS

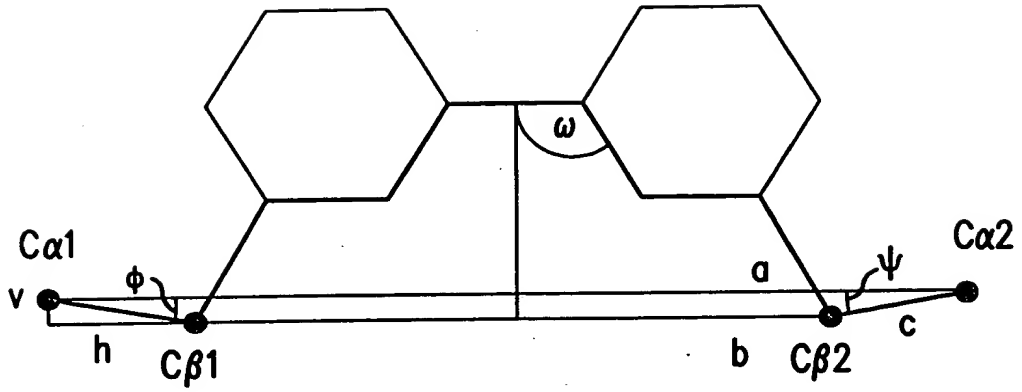

FIG.4A

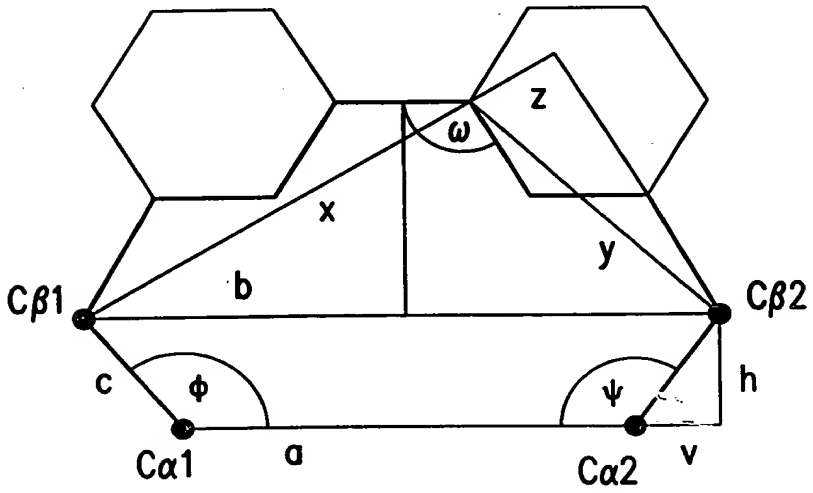

FIG.4B

APPROVED BY SUBCLASS DRAFTS

LIGHT CHAIN (L)

CHAIN	K&W	ATOM	AMINO ACID	x COORDINATE	y COORDINATE	z COORDINATE
L	1	N	ASP	2.37	−5.00	−27.24
L	1	Cα	ASP	2.98	−3.78	−26.64
L	1	C	ASP	1.91	−2.70	−26.52
L	1	O	ASP	1.33	−2.29	−27.53
L	1	Cβ	ASP	4.14	−3.29	−27.53
L	1	Cγ	ASP	5.18	−2.49	−26.76
L	1	Oδ1	ASP	4.86	−1.38	−26.27
L	1	Oδ2	ASP	6.34	−2.97	−26.65
L	2	N	ILE	1.63	−2.26	−25.30
L	2	Cα	ILE	0.60	−1.24	−25.07
L	2	C	ILE	1.19	0.15	−24.94
L	2	O	ILE	2.14	0.35	−24.94
L	2	Cβ	ILE	−0.21	−1.52	−23.78
L	2	Cγ1	ILE	−0.90	−2.88	−23.86
L	2	Cγ2	ILE	−1.24	−0.43	−23.58
L	2	Cδ1	ILE	−1.66	−3.26	−22.59
L	3	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.

FIG.5A

APPROVED BY DRAWING

HEAVY CHAIN (H)

CHAIN	K&W	ATOM	AMINO ACID	x COORDINATE	y COORDINATE	z COORDINATE
H	1	N	GLU	11.12	−2.19	9.00
H	1	Cα	GLU	11.43	−1.08	8.05
H	1	C	GLU	11.93	−1.63	6.71
H	1	O	GLU	13.10	−1.98	6.56
H	1	Cβ	GLU	12.47	−0.12	8.66
H	1	Cγ	GLU	13.82	−0.75	9.05
H	1	Cδ	GLU	13.70	−1.77	10.17
H	1	Oε1	GLU	13.38	−1.36	11.31
H	1	Oε2	GLU	13.94	−2.97	9.92
H	2	N	ILE	11.02	−1.70	5.74
H	2	Cα	ILE	11.36	−2.24	4.42
H	2	C	ILE	12.10	−1.22	3.59
H	2	O	ILE	11.77	−0.04	3.64
H	2	Cβ	ILE	10.11	−2.68	3.62
H	2	Cγ1	ILE	9.31	−3.73	4.39
H	2	Cγ2	ILE	10.52	−3.22	2.28
H	3	Cδ1	ILE	8.49	−3.17	5.55
H	3	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.

FIG.5B

APPROVED BY OTV FTO CLASS

Fv FRAGMENT 1

							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	Cα	Cα	Cα	Cα	Cα
							AA	Asp	Ile	.	.	.
							x	2.98	0.60	.	.	.
							y	−3.78	−1.24	.	.	.
							z	−26.64	−25.07	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	Cα	Glu	11.43	−1.08	8.05		35.80	34.84	.	.	.
H	2	Cα	Ile	11.36	−2.24	4.42		32.21	31.42	.	.	.
H	3	Cα	.	.	.	.		.	.	.	.	.
H	4	Cα	.	.	.	.		.	.	.	.	.
H	5	Cα	.	.	.	.		.	.	.	.	.

FIG.6A

Fv FRAGMENT 2

							Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	Cα	Cα	Cα	Cα	Cα
							AA	Glu	Ser	.	.	.
							x	35.61	31.94	.	.	.
							y	83.10	83.89	.	.	.
							z	56.99	56.85	.	.	.
Ch	K&W	At	AA	x	y	z						
H	1	Cα	Glu	10.23	61.09	64.74		34.48	32.46	.	.	.
H	2	Cα	Val	13.63	62.72	65.19		31.07	29.20	.	.	.
H	3	Cα	.	.	.	.		.	.	.	.	.
H	4	Cα	.	.	.	.		.	.	.	.	.
H	5	Cα	.	.	.	.		.	.	.	.	.

FIG.6B

APPROVED BY DRAFTSMAN

Fv FRAGMENT 3

Ch	K&W	At	AA	x	y	z		Ch	L	L	L	L	L
								K&W	1	2	3	4	5
								At	Cα	Cα	Cα	Cα	Cα
								AA	Glu	Ser	.	.	.
								x	19.56	19.09	.	.	.
								y	−13.02	−15.06	.	.	.
								z	−15.86	−12.67	.	.	.
H	1	Cα	GLN	26.71	9.76	10.88			35.84	35.05	.	.	.
H	2	Cα	Val	27.45	8.61	7.34			32.69	32.11	.	.	.
H	3	Cα	.	.	.	.			.	.	.	.	.
H	4	Cα	.	.	.	.			.	.	.	.	.
H	5	Cα	.	.	.	.			.	.	.	.	.

FIG.6C

ATTORNEY DOCKET NO.
BY
SUBCLASS
DRAFTSMAN

RESIDUE PAIRS		AVERAGE	St.DEV.	MAX	MIN	MEDIAN
H1	L1	35.38	0.78	35.84	34.48	35.80
H1	L2	34.12	1.44	35.05	32.46	34.84
H1	L3	.	.	.	.	.
H1	L4	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
H1	L106	.	.	.	.	.
H2	L1	31.99	0.83	32.69	31.07	32.21
H2	L2	30.91	1.52	32.11	29.20	31.41
H2	L3	.	.	.	.	.
H2	L4	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
H2	L106	.	.	.	.	.
H3	L1	.	.	.	.	.

FIG.7A

RESIDUE PAIRS		AVERAGE	St.DEV.	MAX	MIN	MEDIAN
H1	L1	35.09	1.56	37.37	31.23	35.54
H1	L2	34.00	1.87	37.36	29.92	34.38
H1	L3	.	.	.	.	.
H1	L4	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
H1	L106	.	.	.	.	.
H2	L1	32.26	1.57	36.71	30.34	32.14
H2	L2	31.32	1.99	36.77	29.20	31.11
H2	L3	.	.	.	.	.
H2	L4	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
H2	L106	.	.	.	.	.
H3	L1	.	.	.	.	.

FIG.7B

APPROVED BY DRAFTSMAN

Ch	K&W	At	AA	x	y	z	Ch	L	L	L	L	L
							K&W	1	2	3	4	5
							At	Cβ	Cβ	Cβ	Cβ	Cβ
							AA	ASP	ILE	.	.	.
							x	4.14	−0.21	.	.	.
							y	−3.29	−1.52	.	.	.
							z	−27.53	−23.78	.	.	.
H	1	Cβ	GLU	12.47	−0.12	8.66		37.27	34.85	.	.	.
H	2	Cβ	ILE	10.11	−2.68	3.62		31.73	29.30	.	.	.
H	3	Cβ	.	.	.	.		.	.	.	.	.
H	4	Cβ	.	.	.	.		.	.	.	.	.
H	5	Cβ	.	.	.	.		.	.	.	.	.

FIG.8

ALPHA DISTANCES

Ch	K&W	At	AA	x	y	z	L 1	L 2	L 3	L 4	L 5
							Cα	Cα	Cα	Cα	Cα
							ASP	ILE	.	.	.
							2.98	0.60	.	.	.
							−3.78	−1.24	.	.	.
							−26.64	−25.07	.	.	.
H	1	Cα	GLU	11.43	−1.08	8.05	35.80	34.84	.	.	.
H	2	Cα	ILE	11.36	−2.24	4.42	32.21	31.42	.	.	.
H	3	Cα	.	.	.	.	.	.	.	.	.
H	4	Cα	.	.	.	.	.	.	.	.	.
H	5	Cα	.	.	.	.	.	.	.	.	.

FIG.9A

BETA DISTANCES

Ch	K&W	At	AA	x	y	z	L 1	L 2	L 3	L 4	L 5
							Cβ	Cβ	Cβ	Cβ	Cβ
							ASP	ILE	.	.	.
							4.14	−0.21	.	.	.
							−3.29	−1.52	.	.	.
							−27.53	−23.78	.	.	.
H	1	Cβ	GLU	12.47	−0.12	8.66	37.27	34.85	.	.	.
H	2	Cβ	ILE	10.11	−2.68	3.62	31.73	29.30	.	.	.
H	3	Cβ	.	.	.	.	.	.	.	.	.
H	4	Cβ	.	.	.	.	.	.	.	.	.
H	5	Cβ	.	.	.	.	.	.	.	.	.

FIG.9B

APPROVED BY DRAFTSMAN

DIFFERENCE

DIFFERENCES BETWEEN RESIDUE PAIR
ALPHA- AND BETA CARBON DISTANCES

Ch	K&W	AA	Ch K&W	L 1	L 2	L 3	L 4	L 5
			AA	ASP	ILE	.	.	.
						.	.	.
						.	.	.
						.	.	.
H	1	GLU		-1.47	-0.01	.	.	.
H	2	ILE		0.48	2.10	.	.	.
H	3	.		.	.	.	.	.
H	4	.		.	.	.	.	.
H	5	.		.	.	.	.	.

FIG.9C

APPROVED
BY
DRAFTSMAN

		L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 1	H1	−1.47	−0.01	.	.	.	.	.
	H2	0.48	2.10	.	.	.	.	.
	H3	.	.	.	.	.	.	.
	H4	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.
		L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 2	H1	−1.61	0.46	.	.	.	.	.
	H2	0.18	2.04	.	.	.	.	.
	H3	.	.	.	.	.	.	.
	H3	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.
		L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 3	H1	0.92	1.59	.	.	.	.	.
	H2	0.69	1.31	.	.	.	.	.
	H3	.	.	.	.	.	.	.
	H3	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.
	.	.	.	.	.	.	.	.
		L1	L2	L3	L4	L5	.	.
Fv FRAGMENT 4	H1	.	.	.	.	.	.	.

FIG.10

APPROVED BY DRAFTSMAN

RESIDUE PAIRS		AVERAGE	Strd.DEV.	MAX	MIN	MEDIAN
H1	L1	−0.72	1.42	0.92	−1.61	−1.47
H1	L2	0.68	0.82	1.59	−0.01	0.46
H1	L3	.	.	.	.	.
H1	L4	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
H1	L106	.	.	.	.	.
H2	L1	0.45	0.26	0.69	0.18	0.48
H2	L2	0.68	0.82	1.59	−0.01	0.46
H2	L3	.	.	.	.	.
H2	L4	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
H2	L106	.	.	.	.	.
H3	L1	.	.	.	.	.

FIG.11A

RESIDUE PAIRS		AVERAGE	Strd.DEV.	MAX	MIN	MEDIAN
H1	L1	−0.68	1.04	0.92	−2.20	0.83
H1	L2	0.34	0.82	2.37	−0.54	0.09
H1	L3	.	.	.	.	.
H1	L4	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
H1	L106	.	.	.	.	.
H2	L1	0.74	0.69	1.83	−0.18	0.59
H2	L2	1.78	0.50	2.55	0.75	1.94
H2	L3	.	.	.	.	.
H2	L4	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
H2	L106	.	.	.	.	.
H3	L1	.	.	.	.	.

FIG.11B

APPROVED BY DRAFTSMAN CLASS ASS

Res.	AA	F	AA	F	AA	F	AA	F	AA	F	AA	F
1	Glu	58	Glu	24	Asp	3	Glu	3	Gly	2	Ala	1
2	Val	99	Ile	2	Ala	1	Glu	1	Met	1	–	–
3	Gln	90	Thr	5	Glu	3	His	2	Leu	2	Lys	2
4	Leu	101	Val	3	–	–	–	–	–	–	–	–

FIG.12A

Amino Acid	van der Waals volumes [A^3]	Hydrophobicity
Ala	67	0.62
Arg	148	−2.50
Asn	96	−0.78
Asp	91	−0.90
Cys	86	0.29
Gln	114	−0.85
Glu	109	−0.79
Gly	48	0.30
His	118	−0.40
Ile	124	1.40
Leu	124	1.10
Lys	135	−1.50
Met	124	0.64
Phe	135	1.20
Pro	90	0.12
Ser	73	−0.18
Thr	93	−0.05
Trp	163	0.81
Tyr	141	0.26
Val	105	1.10

FIG.12B

Res.	AA	F	AA	F	AA	F	AA	F	AA	F	AA	F
1	109	61	109	24	91	3	48	2	67	1		
2	105	99	124	2	67	1	109	1	124	1	–	–
3	114	90	93	5	109	3	118	2	124	2	135	2
4	124	101	105	3	–	–	–	–	–	–	–	–

FIG.12C

EP 0 574 948 A2

T09T+0" 9E2/E960

VAN DER WAALS VOLUMES

CHAIN	K&W	CONS.	WEIGHTED AVERAGE	WEIGHTED StDev.	UNWEIGHTED AVERAGE	UNWEIGHTED StDev.
H	1	Glu	108	11	90	27
H	2	Val	105	5	106	23
H	3	Gln	114	6	116	14
H	4	Leu	123	3	115	13

FIG.13A

HYDROPHOBICITY

CHAIN	K&W	CONS.	WEIGHTED AVERAGE	WEIGHTED StDev.	UNWEIGHTED AVERAGE	UNWEIGHTED StDev.
H	1	Glu	−0.77	0.24	−0.37	0.72
H	2	Val	1.08	0.20	0.59	0.84
H	3	Gln	−0.78	0.33	−0.42	0.89
H	4	Leu	1.10	0.00	1.10	0.00

FIG.13B

APPROVED BY CLASS

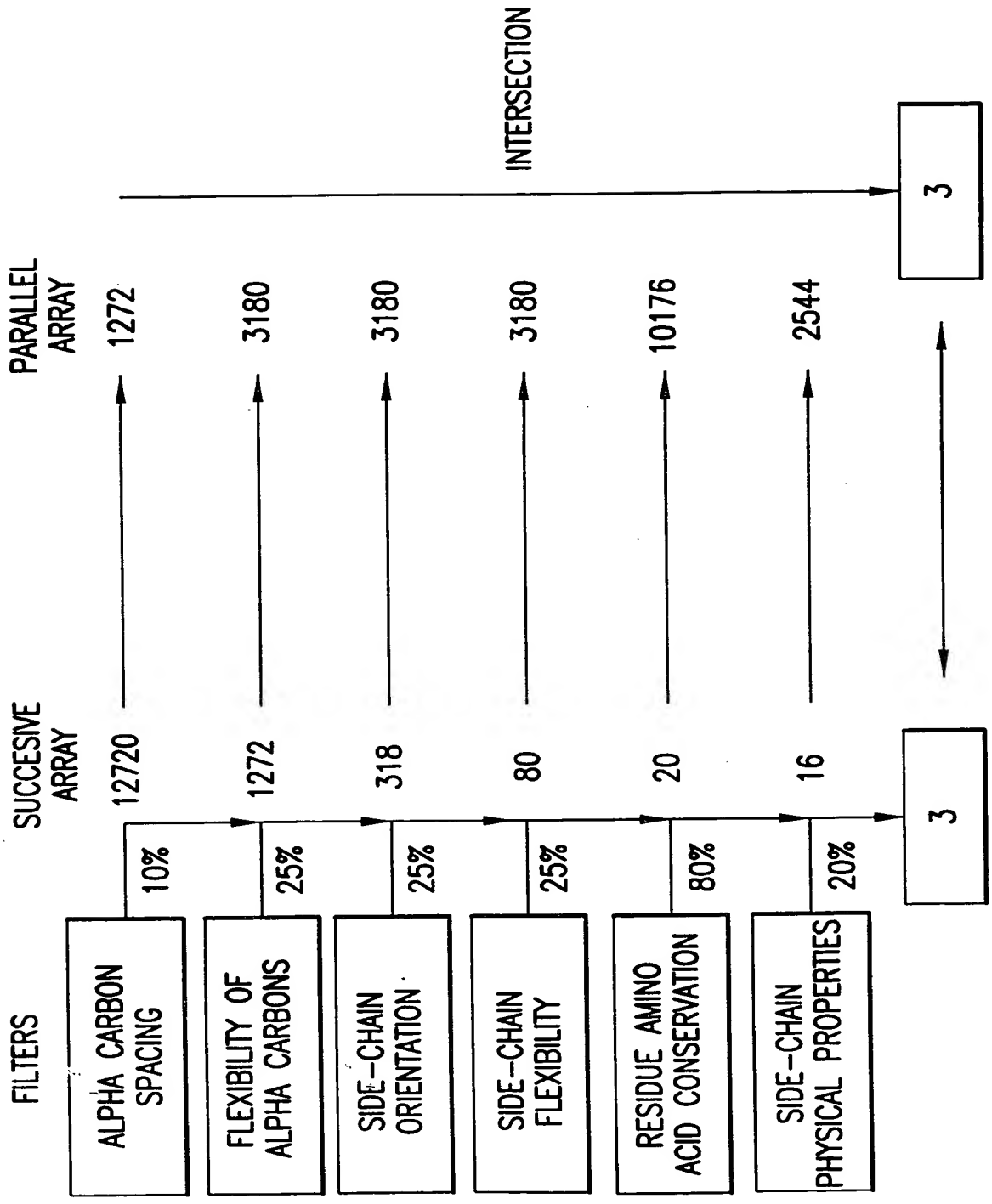

FIG.14

APPROVED BY ... ISS

C. Antarctica Lipase B Nucleotide and Amino Acid Sequence

```
        10         20         30         40         50         60
ctaccttccggttcggaccctgccttttcgcagcccaagtcggtgctcgatgcgggtctg
 L  P  S  G  S  D  P  A  F  S  Q  P  K  S  V  L  D  A  G  L
        70         80         90        100        110        120
acctgccagggtgcttcgccatcctcggtctccaaacccatccttctcgtccccggaacc
 T  C  Q  G  A  S  P  S  S  V  S  K  P  I  L  L  V  P  G  T
       130        140        150        160        170        180
ggcaccacaggtccacagtcgttcgactcgaactggatccccctctcaacgcagttgggt
 G  T  T  G  P  Q  S  F  D  S  N  W  I  P  L  S  T  Q  L  G
       190        200        210        220        230        240
tacacaccctgctggatctcacccccgccgttcatgctcaacgacacccaggtcaacacg
 Y  T  P  C  W  I  S  P  P  P  F  M  L  N  D  T  Q  V  N  T
       250        260        270        280        290        300
gagtacatggtcaacgccatcaccgcgctctacgctggttcgggcaacaacaagcttccc
 E  Y  M  V  N  A  I  T  A  L  Y  A  G  S  G  N  N  K  L  P
       310        320        330        340        350        360
gtgcttacctggtcccagggtggtctggttgcacagtggggtctgaccttcttccccagt
 V  L  T  W  S  Q  G  G  L  V  A  Q  W  G  L  T  F  F  P  S
       370        380        390        400        410        420
atcaggtccaaggtcgatcgacttatggcctttgcgcccgactacaagggcaccgtcctc
 I  R  S  K  V  D  R  L  M  A  F  A  P  D  Y  K  G  T  V  L
       430        440        450        460        470        480
gccggccctctcgatgcactcgcggttagtgcaccctccgtatggcagcaaaccaccggt
 A  G  P  L  D  A  L  A  V  S  A  P  S  V  W  Q  Q  T  T  G
       490        500        510        520        530        540
tcggcactcaccaccgcactccgaaacgcaggtggtctgacccagatcgtgcccaccacc
 S  A  L  T  T  A  L  R  N  A  G  G  L  T  Q  I  V  P  T  T
       550        560        570        580        590        600
aacctctactcggcgaccgacgagatcgttcagcctcaggtgtccaactcgccactcgac
 N  L  Y  S  A  T  D  E  I  V  Q  P  Q  V  S  N  S  P  L  D
       610        620        630        640        650        660
tcatcctacctcttcaacggaaagaacgtccaggcacaggccgtgtgtgggccgctgttc
 S  S  Y  L  F  N  G  K  N  V  Q  A  Q  A  V  C  G  P  L  F
       670        680        690        700        710        720
gtcatcgaccatgcaggctcgctcacctcgcagttctcctacgtcgtcggtcgatccgcc
 V  I  D  H  A  G  S  L  T  S  Q  F  S  Y  V  V  G  R  S  A
       730        740        750        760        770        780
ctgcgctccaccacgggccaggctcgtagtgcagactatggcattacggactgcaaccct
 L  R  S  T  T  G  Q  A  R  S  A  D  Y  G  I  T  D  C  N  P
       790        800        810        820        830        840
cttcccgccaatgatctgactcccgagcaaaaggtcgccgcggctgcgctcctggcgccg
 L  P  A  N  D  L  T  P  E  Q  K  V  A  A  A  A  L  L  A  P
       850        860        870        880        890        900
gcagctgcagccatcgtggcgggtccaaagcagaactgcgagcccgacctcatgccctac
 A  A  A  A  I  V  A  G  P  K  Q  N  C  E  P  D  L  M  P  Y
       910        920        930        940        950
gcccgccccttgcagtaggcaaaaggacctgctccggcatcgtcacccccctga
 A  R  P  F  A  V  G  K  R  T  C  S  G  I  V  T  P  *
```

Docket No. 9725-005

FIG. 15A

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PCR Oligos for *Candida antarctica* Lipase B

Oligos for pPal-CALB

Primer A: 5'atg gga att cca tca tca tca tca cag cag cgg cct acc ttc cgg ttc gga ccc3'

Primer B: 5'ctc ttg gcg gcc gcc tat cag ggg gtg acg atg ccg g3'

Oligos for Point Mutations (made in pPal-CALB)

M1- F9Y

primer M1F: 5'atg gga att cca tca tca tca tca cag cag cgg cct acc ttc cgg ttc gga ccc tgc ctA ttc gc3'

M2- W52Y

Primer M2F: 5'cga ctc gaa ctA Cat ccc cct ctc3'

Primer M2R: 5'gag agg ggg atG Tag ttc gag tcg3'

M3- F117Y

Primer M3F: 5'ggg tctg acc tAc ttc ccc agt atc3'

Primer M3R: 5'gat act ggg gaa gTa ggt cag acc c3'

Oligos for pYal-CALB

Primer C:

5'- cgA Tga gat ttc ctt caa ttt -3'

Primer D:

5'-5'tct aga aag gtg gcg gcc gcc -3'

Oligos for error-prone PCR

Primer E:

5'gaa gct gga ttc cat cat cat c3'

Primer D:

5'-5'tct aga aag gtg gcg gcc gcc -3'

FIG. 15B

APPROVED BY DRAFTSMAN
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CLASS SUBCL.

Subtilisin E Nucleotide and Amino Acid Sequence

```
        10         20         30         40         50         60         70         80
atgtctgtgcaggctgccggaaaaagcagtacagaaaagaaatacattgtcggatttaaacagacaatgagtgccatgag
 M  S  V  Q  A  A  G  K  S  S  T  E  K  K  Y  I  V  G  F  K  Q  T  M  S  A  M  S
        90         100        110        120        130        140        150        160
ttccgccaagaaaaaggatgttatttctgaaaaaggcggaaaggttcaaaagcaatttaagtatgttaacgcggccgcag
  S  A  K  K  K  D  V  I  S  E  K  G  G  K  V  Q  K  Q  F  K  Y  V  N  A  A  A
       170        180        190        200        210        220        230        240
caacattggatgaaaaagctgtaaaagaattgaaaaaagatccgagcgttgcatatgtggaagaagatcatattgcacat
A  T  L  D  E  K  A  V  K  E  L  K  K  D  P  S  V  A  Y  V  E  E  D  H  I  A  H
       250        260        270        280        290        300        310        320
gaatatgcgcaatctgttccttatggcatttctcaaattaaagcgccggctcttcactctcaaggctacacaggctctaa
 E  Y  A  Q  S  V  P  Y  G  I  S  Q  I  K  A  P  A  L  H  S  Q  G  Y  T  G  S  N
       330        340        350        360        370        380        390        400
cgtaaaagtagctgttatcgacagcggaattgactcttctcatcctgacttaaacgtcagaggcggagcaagcttcgtac
  V  K  V  A  V  I  D  S  G  I  D  S  S  H  P  D  L  N  V  R  G  G  A  S  F  V
       410        420        430        440        450        460        470        480
cttctgaaacaaacccataccaggacggcagttctcacggtacgcatgtagccggtacgattgccgctcttaataactca
P  S  E  T  N  P  Y  Q  D  G  S  S  H  G  T  H  V  A  G  T  I  A  A  L  N  N  S
       490        500        510        520        530        540        550        560
atcggtgttctgggcgttagcccaagcgcatcattatatgcagtaaaagtgcttgattcaacaggaagcggccaatatag
 I  G  V  L  G  V  S  P  S  A  S  L  Y  A  V  K  V  L  D  S  T  G  S  G  Q  Y  S
       570        580        590        600        610        620        630        640
ctggattattaacggcattgagtgggccatttccaacaatatggatgttatcaacatgagccttggcggacctactggtt
  W  I  I  N  G  I  E  W  A  I  S  N  N  M  D  V  I  N  M  S  L  G  G  P  T  G
       650        660        670        680        690        700        710        720
ctacagcgctgaaaacagtcgttgacaaagccgtttccagcggtatcgtcgttgctgccgcagccggaaacgaaggttca
S  T  A  L  K  T  V  V  D  K  A  V  S  S  G  I  V  V  A  A  A  A  G  N  E  G  S
       730        740        750        760        770        780        790        800
tccggaagcacaagcacagtcggctaccctgcaaaatatccttctactattgcagtaggtgcggtaaacagcagcaacca
 S  G  S  T  S  T  V  G  Y  P  A  K  Y  P  S  T  I  A  V  G  A  V  N  S  S  N  Q
       810        820        830        840        850        860        870        880
aagagcttcattctccagcgcaggttctgagcttgatgtgatggctcctggcgtgtccatccaaagcacacttcctggag
  R  A  S  F  S  S  A  G  S  E  L  D  V  M  A  P  G  V  S  I  Q  S  T  L  P  G
       890        900        910        920        930        940        950        960
gcacttacggcgcttataacggaacgtccatggcgactcctcacgttgccggagcagcagcgttaattctttctaagcac
G  T  Y  G  A  Y  N  G  T  S  M  A  T  P  H  V  A  G  A  A  A  L  I  L  S  K  H
       970        980        990       1000       1010       1020       1030       1040
ccgacttggacaaacgcgcaagtccgtgatcgtttagaaagcactgcaacatatcttggaaactctttctactatggaaa
 P  T  W  T  N  A  Q  V  R  D  R  L  E  S  T  A  T  Y  L  G  N  S  F  Y  Y  G  K
      1050       1060       1070
agggttaatcaacgtacaagcagctgcacaataa
  G  L  I  N  V  Q  A  A  A  Q  *
```

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FIG. 16A

APPROVED O.I.P.E.
BY CLASS SUBCLASS

Subtilisin Amino Acid Alignment

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ALA	GLN	SER	VAL	PRO	TRP	GLY	ILE	SER	ARG	VAL	GLN	ALA	PRO	ALA	ALA	HIS	ASN
ALA	GLN	SER	VAL	PRO	TYR	GLY	ILE	SER	GLN	ILE	LYS	ALA	PRO	ALA	LEU	HIS	SER
ALA	LYS	CYS	VAL	SER	TYR	GLY	VAL	SER	GLN	ILE	LYS	ALA	PRO	ALA	LEU	HIS	SER

19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
ARG	GLY	LEU	THR	GLY	SER	GLY	VAL	LYS	VAL	ALA	VAL	LEU	ASP	THR	GLY	ILE	SER
GLN	GLY	TYR	THR	GLY	SER	ASN	VAL	LYS	VAL	ALA	VAL	ILE	ASP	SER	GLY	ILE	ASP
GLN	GLY	TYR	THR	GLY	SER	ASN	VAL	LYS	VAL	ALA	VAL	ILE	ASP	SER	GLY	ILE	ASP

37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
THR	---	HIS	PRO	ASP	LEU	ASN	ILE	ARG	GLY	GLY	ALA	SER	PHE	VAL	PRO	GLY	GLU
SER	SER	HIS	PRO	ASP	LEU	ASN	VAL	ARG	GLY	GLY	ALA	SER	PHE	VAL	PRO	SER	GLU
SER	SER	HIS	PRO	ASP	LEU	ASN	VAL	ALA	GLY	GLY	ALA	SER	PHE	VAL	PRO	SER	GLU

55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
---	---	PRO	SER	THR	GLN	ASP	GLY	ASN	GLY	HIS	GLY	THR	HIS	VAL	ALA	GLY	THR
THR	ASN	PRO	TYR	---	GLN	ASP	GLY	SER	SER	HIS	GLY	THR	HIS	VAL	ALA	GLY	THR
THR	ASN	PRO	PHE	---	GLN	ASP	ASN	ASN	SER	HIS	GLY	THR	HIS	VAL	ALA	GLY	THR

73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
ILE	ALA	ALA	LEU	ASN	ASN	SER	ILE	GLY	VAL	LEU	GLY	VAL	ALA	PRO	ASN	ALA	GLU
ILE	ALA	ALA	LEU	ASN	ASN	SER	ILE	GLY	VAL	LEU	GLY	VAL	SER	PRO	SER	ALA	SER
---	---	---	---	---	---	---	---	---	VAL	LEU	ALA	VAL	ALA	PRO	SER	ALA	SER

91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
LEU	TYR	ALA	VAL	LYS	VAL	LEU	GLY	ALA	SER	GLY	SER	GLY	SER	VAL	SER	SER	ILE
LEU	TYR	ALA	VAL	LYS	VAL	LEU	ASP	SER	THR	GLY	SER	GLY	GLN	TYR	SER	TRP	ILE
LEU	TYR	ALA	VAL	LYS	VAL	LEU	GLY	ALA	ASP	GLY	SER	GLY	GLN	TYR	SER	TRP	ILE

109	110	111	112	113	114	115	116	117	118	118	120	121	122	123	124	125	126
ALA	GLN	GLY	LEU	GLU	TRP	ALA	GLY	ASN	ASN	GLY	MET	HIS	VAL	ALA	ASN	LEU	SER
ILE	ASN	GLY	ILE	GLU	TRP	ALA	ILE	SER	ASN	ASN	MET	ASP	VAL	ILE	ASN	MET	SER
ILE	ASN	GLY	ILE	GLU	TRP	ALA	ILE	ALA	ASN	ASN	MET	ASP	VAL	ILE	ASN	MET	SER

127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144
LEU	GLY	SER	PRO	SER	PRO	SER	ALA	THR	LEU	GLU	GLN	ALA	VAL	ASN	SER	ALA	THR
LEU	GLY	GLY	PRO	THR	GLY	SER	THR	ALA	LEU	LYS	THR	VAL	VAL	ASP	LYS	ALA	VAL
LEU	GLY	GLY	PRO	SER	GLY	SER	ALA	ALA	LEU	LYS	ALA	ALA	VAL	ASP	LYS	ALA	VAL

145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162
SER	ARG	GLY	VAL	LEU	VAL	VAL	ALA	ALA	SER	GLY	ASN	SER	GLY	---	ALA	GLY	SER
SER	SER	GLY	ILE	VAL	VAL	ALA	ALA	ALA	ALA	GLY	ASN	GLU	GLY	SER	SER	GLY	SER
ALA	SER	GLY	VAL	VAL	VAL	VAL	ALA	ALA	ALA	GLY	ASN	GLU	GLY	THR	SER	GLY	SER

FIG. 16B

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Subtilisin Amino Acid Alignment (cont'd.)

```
163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
ILE SER --- --- --- TYR PRO ALA ARG TYR ALA ASN ALA MET ALA VAL GLY ALA
THR SER THR VAL GLY TYR PRO ALA LYS TYR PRO SER THR ILE ALA VAL GLY ALA
SER SER THR VAL GLY TYR PRO GLY LYS TYR PRO SER VAL ILE ALA VAL GLY ALA

181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198
THR ASP GLN ASN ASN ASN ARG ALA SER PHE SER GLN TYR GLY ALA GLY LEU ASP
VAL ASN SER SER ASN GLN ARG ALA SER PHE SER SER ALA GLY SER GLU LEU ASP
VAL ASP SER SER ASN GLN ARG ALA SER PHE SER SER VAL GLY PRO GLU LEU ASP

199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216
ILE VAL ALA PRO GLY VAL ASN VAL GLN SER THR TYR PRO GLY SER THR TYR ALA
VAL MET ALA PRO GLY VAL SER ILE GLN SER THR LEU PRO GLY GLY THR TYR GLY
VAL MET ALA PRO GLY VAL SER ILE CYS SER THR LEU PRO GLY ASN LYS TYR GLY

217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234
SER LEU ASN GLY THR SER MET ALA THR PRO HIS VAL ALA GLY ALA ALA ALA LEU
ALA TYR ASN GLY THR CYS MET ALA THR PRO HIS VAL ALA GLY ALA ALA ALA LEU
ALA LYS SER GLY THR SER MET ALA SER PRO HIS VAL ALA GLY ALA ALA ALA LEU

235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252
VAL LYS GLN LYS ASN PRO SER TRP SER ASN VAL GLN ILE ARG ASN HIS LEU LYS
ILE LEU SER LYS HIS PRO THR TRP THR ASN ALA GLN VAL ARG ASP ARG LEU GLU
ILE LEU SER LYS HIS PRO ASN TRP THR ASN THR GLN VAL ARG SER SER LEU GLU

253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
ASN THR ALA THR SER LEU GLY SER THR ASN LEU TYR GLY SER GLY LEU VAL ASN
SER THR ALA THR TYR LEU GLY ASN SER PHE TYR TYR GLY LYS GLY LEU ILE ASN
ASN THR THR THR LYS LEU GLY ASN SER PHE TYR TYR GLY LYS GLY LEU ILE ASN

271 272 273 274 275 276
ALA GLU ALA ALA THR ARG
VAL GLN ALA ALA ALA GLN
VAL GLN ALA ALA ALA GLN
```

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FIG. 16C

APPROVED BY DRAFTSMAN | C. . FIG. | CLASS | SUBCLASS

PCR Oligos for Subtilisin E

A primer-

5'-ccg agc gttg cat atg tgg aag-3'

B-primer-

5'-tta gga tcc tta atg atg atg atg atg atg ttg tgc agc tgc ttg tac gtt gat-3'

1- K27Y

F 5'-ggc tct aac gta TaT gta gct gtt atc-3'

R 5'-gat aac agc tac AtA tac gtt aga gcc-3'

2- K237Y

F 5'-tta att ctt tct TaC cac ccg act tgg-3'

R 5'-cca agt cgg gtg GtA aga aag aat taa c-3'

3.1- D36Y

F 5'-gac agc gga att T act ctt ctc atc-3'

R 5'-gat gag aag agt A aat tcc gct gtc-3'

3.2- P210Y

F 5'-caa agc aca ctt TAt gga ggc act tac-3'

R 5'-ta agt gcc tcc aTA aag tgt gct ttg-3'

4.1- K170Y

F 5'-ggc tac cct gca TaT tat cct tct act a-3'

R 5'-agt aga agg ata AtA tgc agg gta gcc-3'

4.2- E195Y

F 5'-agc gca ggt tct TaT ctt gat gtg atg -3'

R 5'-cat cac atc aag AtA aga acc tgc gct-3'

5.1- G61Y

F 5'-cca tac cag gac TAc agt tct cac gg-3'

R 5'-cc gtg aga act gTA gtc ctg gta tgg-3'

5.2- S98Y

F 5'-aa gtg ctt gat TAT aca gga agc ggc-3'

R 5'-gcc gct tcc tgt ATA atc aag cac tt-3'

6.1- H17Y

F 5'-gcg ccg gct ctt Tac tct caa ggc t-3'

R 5'-a gcc ttg aga gtA aag agc cgg cgc-3'

6.2- P86Y

F 5'-ctg ggc gtt agc TAT agc gca tca tta-3'

R 3'-taa tga tgc gct ATA gct aac gcc cag-3'

7- P201Y

F 5'-gat gtg atg gct TAt ggc gtg tcc atc-3'

R 5'-gat gga cac gcc aTA agc cat cac atc-3'

FIG. 16D